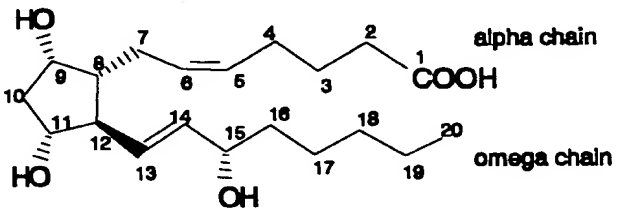
USE OF CLOPROSTENOL AND FLUPROSTENOL ANALOGUES TO TREAT GLAUCOMA AND OCULAR HYPERTENSION

The present application is a continuation-in-part of U.S. Patent Application Serial Number 08/101,598 filed August 3, 1993, now U.S. Patent No. 5,510,383

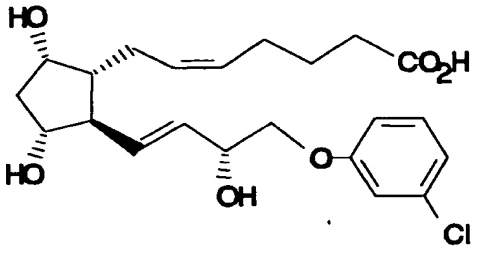
BACKGROUND OF THE INVENTION

The present invention relates to the treatment of glaucoma and ocular hypertension. In particular, the present invention relates to the use of cloprostenol and fluprostenol analogues for the treatment of glaucoma and ocular hypertension.

Cloprostenol and fluprostenol, both known compounds, are synthetic analogues of $PGF_{2\alpha}$, a naturally-occurring F-series prostaglandin (PG). Structures for $PGF_{2\alpha}$ (I), cloprostenol (II), and fluprostenol (III), are shown below:



(I)



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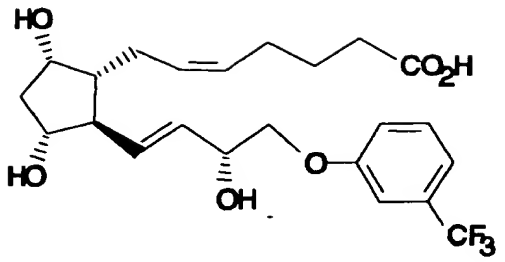


(III)

The chemical name for cloprostenol is 16-(3-chlorophenoxy)-17,18,19,20-tetranor $PGF_{2\alpha}$. Monograph No. 2397 (page 375) of The Merck Index, 11th Edition (1989) is incorporated herein by reference to the extent that it describes the preparation and known pharmacological profiles of cloprostenol. Fluprostenol has the chemical name 16-(3-trifluoromethylphenoxy)-17,18,19,20-tetranor $PGF_{2\alpha}$. Monograph No. 4121 (pages 656-657) of The Merck Index, 11th Edition (1989) is incorporated herein by reference to the extent that it describes the preparation and known pharmacological profiles of fluprostenol. Cloprostenol and fluprostenol are 16-aryloxy PGs and, in addition to the substituted aromatic ring, differ from the natural product $PGF_{2\alpha}$ in that an oxygen atom is embedded within the lower (omega) chain. This oxygen interruption forms an ether functionality.

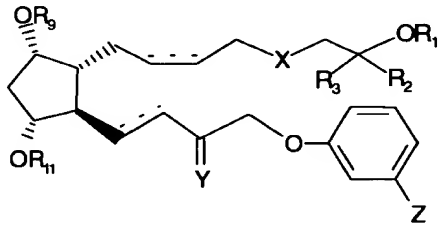
Naturally-occurring prostaglandins are known to lower intraocular pressure (IOP) after topical ocular instillation, but generally cause inflammation, as well as surface irritation characterized by conjunctival hyperemia and edema. Many synthetic prostaglandins have been observed to lower intraocular pressure, but such compounds also produce the aforementioned side effects which severely restrict clinical utility.

SUMMARY OF THE INVENTION

It has now been unexpectedly found that certain novel cloprostenol and fluprostenol analogues are useful in treating glaucoma and ocular hypertension. In particular, topical application of ophthalmic compositions comprising these novel cloprostenol and fluprostenol analogues result in significant IOP reduction.

DETAILED DESCRIPTION OF THE INVENTION

The compounds useful in the present invention have the following general formula:



(IV)

wherein:

R_1 = H; C_1-C_{12} straight-chain or branched alkyl; C_1-C_{12} straight-chain or branched acyl; C_3-C_8 cycloalkyl; or a cationic salt moiety;

R_2, R_3 = H, or C_1-C_5 straight-chain or branched alkyl; or R_2 and R_3 taken together may represent O;

X = O, S, or CH_2;

---- represents any combination of a single bond, or a *cis* or *trans* double bond for the alpha (upper) chain; and a single bond or *trans* double bond for the omega (lower) chain;

R_9 = H, C_1-C_{10} straight-chain or branched alkyl, or C_1-C_{10} straight-chain or branched acyl;

R_{11} = H, C_1-C_{10} straight-chain or branched alkyl, or C_1-C_{10} straight-chain or branched acyl;

Y = O; or H and OR_{15} in either configuration wherein R_{15} = H, C_1-C_{10} straight-chain or branched alkyl, or C_1-C_{10} straight-chain or branched acyl; and

Z = Cl or CF_3;

with the proviso that when R_2 and R_3 taken together represent O, then $R_1 \neq C_1$-C_{12} straight-chain or branched acyl; and when $R_2 = R_3$ = H, then $R_1 \neq$ a cationic salt moiety.

As used herein, the term "cationic salt moiety" includes alkali and alkaline earth metal salts as well as ammonium salts.

Preferred compounds include the 3-oxa form of cloprostenol isopropyl ester (Table, 1, compound **5**), 13,14-dihydrofluprostenol isopropyl ester (compound **6**), cloprostenol-1-ol (compound **7**), and 13,14-dihydrocloprostenol-1-ol pivaloate (compound **8**).

The compounds of formula (IV) are useful in lowering intraocular pressure and thus are useful in the treatment of glaucoma. The preferred route of administration is topical. The dosage range for topical administration is generally between about 0.01 and about 1000 micrograms per eye (μg/eye), preferably between about 0.1 and about 100 μg/eye, and most preferably between about 1 and 10 μg/eye. The compounds of the present invention can be administered as solutions, suspensions, or emulsions (dispersions) in a suitable ophthalmic vehicle.

In forming compositions for topical administration, the compounds of the present invention are generally formulated as between about 0.00003 to about 3 percent by weight (wt%) solutions in water at a pH between 4.5 to 8.0. The compounds are preferably formulated as between about 0.0003 to about 0.3 wt% and, most preferably, between about 0.003 and about 0.03 wt%. While the precise

regimen is left to the discretion of the clinician, it is recommended that the resulting solution be topically applied by placing one drop in each eye one or two times a day.

Other ingredients which may be desirable to use in the ophthalmic preparations of the present invention include preservatives, co-solvents and viscosity building agents.

Antimicrobial Preservatives:

Ophthalmic products are typically packaged in multidose form, which generally require the addition of preservatives to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, ONAMER M®, or other agents known to those skilled in the art. Such preservatives are typically employed at a concentration between about 0.001% and about 1.0% by weight.

Co-Solvents:

Prostaglandins, and particularly ester derivatives, typically have limited solubility in water and therefore may require a surfactant or other appropriate co-solvent in the composition. Such co-solvents include: Polysorbate 20, 60 and 80; Pluronic F-68, F-84 and P-103; Tyloxapol ®; Cremophor® EL; sodium dodecyl sulfate; glycerol; PEG 400; propylene glycol; cyclodextrins; or other agents known to those skilled in the art. Such co-solvents are typically employed at a concentration between about 0.01% and about 2% by weight.

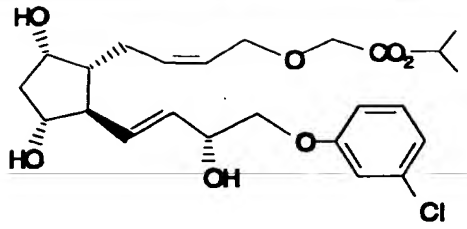
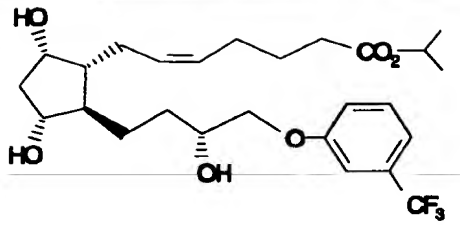
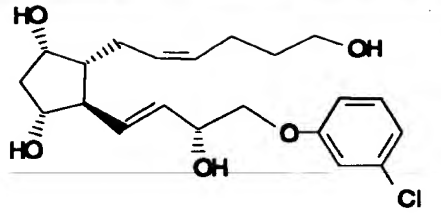
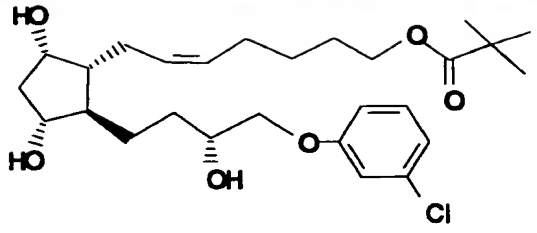
Viscosity Agents:

Viscosity greater than that of simple aqueous solutions may be desirable to increase ocular absorption of the active compound, to decrease variability in dispensing the formulations, to decrease physical separation of components of a suspension or emulsion of formulation and/or otherwise to improve the ophthalmic

formulation. Such viscosity building agents include, for example, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose or other agents known to those skilled in the art. Such agents are typically employed at a concentration between about 0.01% and about 2% by weight.

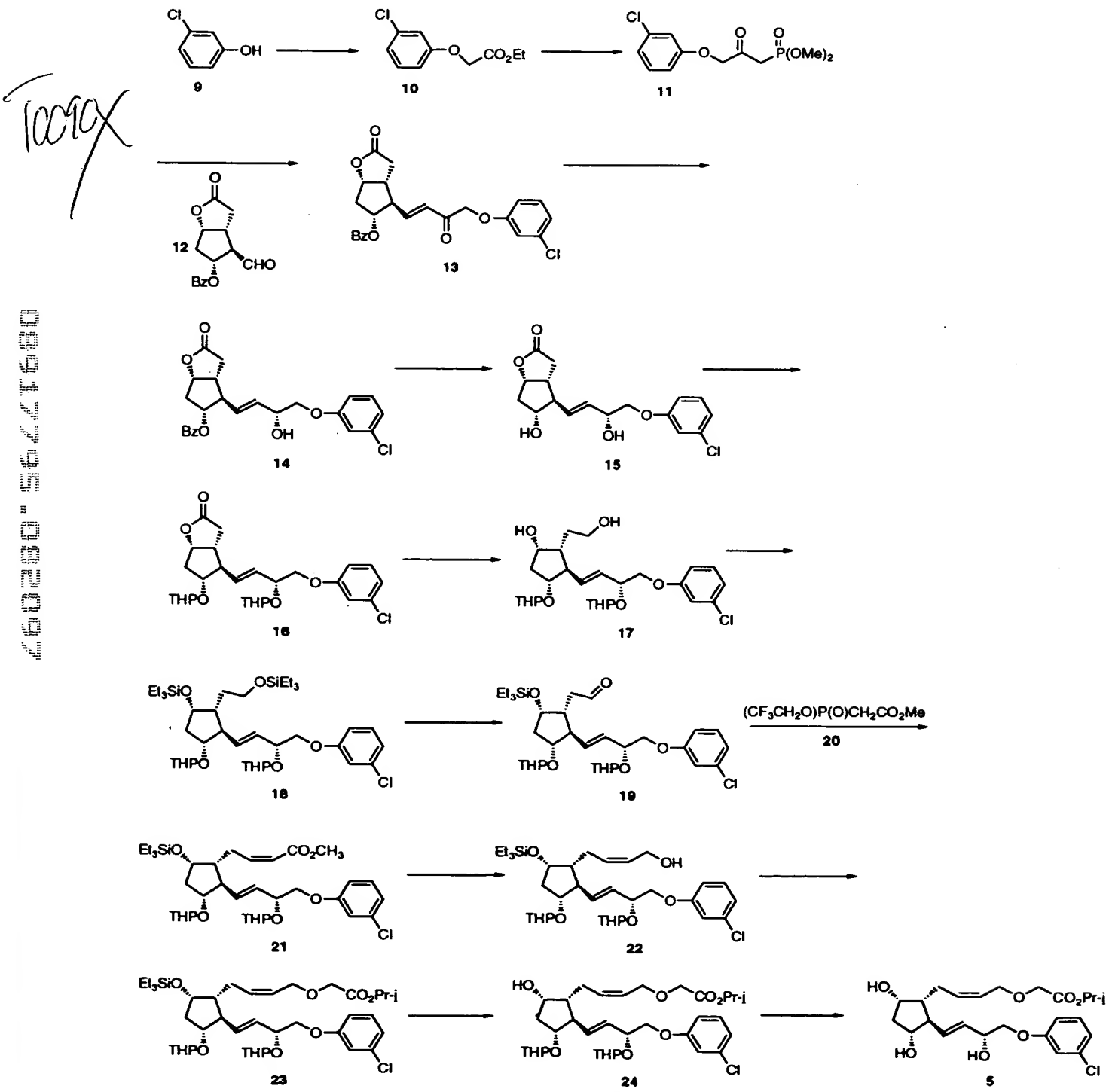
The following Examples 1-4 describe the synthesis of compounds **5-8** (Table 1). These syntheses are representative in nature and are not intended to be limiting. Other compounds of formula (IV) may be prepared using analogous techniques known to those skilled in the art.

Table 1

	COMPOUND NAME	COMPOUND STRUCTURE
5	3-oxacloprostenol isopropyl ester	
6	13,14-dihydrofluprostenol isopropyl ester	
7	cloprostenol-1-ol	
8	13,14-dihydrocloprostenol-1-ol pivaloate	

In the examples below, the following standard abbreviations are used: g = grams (mg = milligrams); mol = moles (mmol = millimoles); mol% = mole percent; mL = milliliters; mm Hg = millimeters of mercury; mp = melting point; bp = boiling point; h = hours; and min = minutes. In addition, "NMR" refers to nuclear magnetic resonance spectroscopy and "CI MS" refers to chemical ionization mass spectrometry.

EXAMPLE 1: Synthesis of 3-Oxacloprostenol (5)



A: Ethyl (3-chlorophenoxy)acetate (**10**)

Acetone (320 ml), 75 g (450 mmol) of ethyl bromoacetate, and 40.0 g (310 mmol) of 3-chlorophenol were mixed together, then 69.8 g (505 mmol) of potassium carbonate was added. The mixture was mechanically stirred and heated to reflux for 4 h, and after cooling to room temperature, was poured into 350 mL of ethyl acetate. To this was then cautiously added 400 mL of 1 *M* HCl, taking care to avoid excess foaming. The layers were separated and the aqueous layer was extracted with portions of ethyl acetate (3 X 200 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and the resulting solid was recrystallized from hexane to afford 58 g (87%) of **10** as a white solid, m.p. = 39-40 °C. ^{1}H NMR δ 7.20-7.08 (m, 1 H), 6.95-6.82 (m, 2 H), 6.75-6.70 (m, 1 H), 4.53 (s, 2 H), 4.21 (q, J = 7.2 Hz, 2 H), 1.23 (t, J = 7.2 Hz, 3 H).

B: Dimethyl [3-(3-chlorophenoxy)-2-oxoprop-1-yl]phosphonate (**11**)

To 20.6 g (166 mmol, 238 mol%) of dimethyl methylphosphonate in 110 mL of THF at -78 °C was added dropwise 65 mL (162 mmol, 232 mol%) of a 2.5 *M* solution of *n*-BuLi in hexanes. After addition was complete, the mixture was stirred for an additional 1 h, after which 15.0 g (69.9 mmol) of aryloxyester **10** in 40 mL of THF was added dropwise. The reaction was stirred for 1 h and then quenched by the addition of 100 mL of saturated NH_4Cl. The mixture was poured into 200 mL of a 1/1 mixture of saturated NaCl/ethyl acetate, layers were separated, and the aqueous layer was further extracted with ethyl acetate (2 X 100 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated, to afford 20.5 g (100%) of **11** as a viscous oil. ^{1}H NMR δ 7.22 (t, J = 8.1 Hz, 1 H), 7.05-6.90 (m, 2 H), 6.85-6.78 (m, 1 H), 4.72 (s, 2 H), 3.84 (s, 3 H), 3.78 (s, 3 H), 3.27 (d, J = 22.8 Hz, 2 H).

10

C: (3a*R*, 4*R*, 5*R*, 6a*S*)-5-(Benzoyloxy)-4-[(*E*)-4-(3-chlorophenoxy)-3-oxo-1-butenyl]-hexahydro-2*H*-cyclopenta[b]furan-2-one (**13**)

Phosphonate **11** (20.5 g, 70.0 mmol), 2.6 g (62 mmol) of LiCl, and 200 mL of THF were mixed together at 0 °C and 6.10 g (60.4 mmol) of NEt_3 was added. Aldehyde **12** (14.0 g, 51.1 mmol) dissolved in 50 mL of CH_2Cl_2 was then added dropwise. After 1 h, the reaction was poured into 200 mL of a 1/1 mixture of saturated NH_4Cl/ethyl acetate, the layers were separated, and the aqueous layer was extracted with ethyl acetate (2 X 100 mL). Combined organic layers were dried over $MgSO_4$, filtered, concentrated, and the residue was chromatographed on silica gel eluting with ethyl acetate/hexanes, 3/2, to afford 16.2 g (72%) of **13** as a white crystalline solid, m.p. = 101.0-102.0 °C. ^1H NMR δ 8.0-7.9 (m, 2 H), 7.62-7.52 (m, 1 H), 7.50-7.38 (m, 2 H), 7.18 (t, J = 8.2 Hz, 1 H), 7.0-6.82 (m, 3 H), 6.75-6.70 (m, 1 H), 6.54 (d, J = 15.1 Hz, 1 H), 5.32 (q, J = 6.2 Hz, 1 H), 5.12-5.05 (m, 1 H), 4.66 (s, 2 H), 3.0-2.8 (m, 3 H), 2.7-2.2 (m, 3 H).

D: (3a*R*, 4*R*, 5*R*, 6a*S*)-5-(Benzoyloxy)-4-[(*E*)-(3*R*)-4-(3-chlorophenoxy)-3-hydroxy-1-butenyl]-hexahydro-2*H*-cyclopenta[b]furan-2-one (**14**)

To a solution of 9.70 g (22.0 mmol) of enone **13** in 60 mL of THF at -23 °C was added dropwise a solution of 11.1 g (34.6 mmol) of (-)-*B*-chlorodiisopinocampheylborane in 30 mL of THF. After 4 h, the reaction was quenched by the dropwise addition of 5 mL of methanol and then warmed to room temperature. After pouring into 200 mL of a 2/1 mixture of ethyl acetate/saturated NH_4Cl, the layers were separated, and the aqueous phase was extracted with ethyl acetate (2 X 100 mL). Combined organic layers were dried over $MgSO_4$, filtered, concentrated, and the residue was chromatographed on silica gel eluting with ethyl acetate/hexanes, 3/2, to afford 4.7 g (48%) of **14** as a white solid, m. p. 101.0-102.5 °C. ^1H NMR δ 8.05-7.95 (m, 2 H), 7.62-7.40 (m, 3 H), 7.18 (t, J = 8.0 Hz, 1 H), 7.0-6.92 (m, 1 H), 6.85 (t, J = 2.1 Hz, 1 H), 6.77-6.70 (m, 1 H), 5.85 (d of d, J = 6.2, 15.5 Hz, 1 H), 5.72 (d of d, J = 4.5, 15.5 Hz, 1 H), 5.30 (q, J = 5.8 Hz, 1 H), 5.12-5.04 (m, 1 H), 4.58-4.48 (m, 1 H), 3.92 (d of d, J = 3.5, 9.3 Hz, 1 H), 3.80 (d of d, J = 7.3, 9.4 Hz, 1 H), 2.9-2.2 (m, 8 H).

E: (3a*R*, 4*R*, 5*R*, 6a*S*)-4-[(*E*)-(3*R*)-4-(3-Chlorophenoxy)-3-(tetrahydropyran-2-yloxy)-1-butenyl]-5-(tetrahydropyran-2-yloxy)-hexahydro-2*H*-cyclopenta[b]furan-2-one (**16**)

To a mixture of 5.1 g (11.5 mmol) of **14** in 200 mL of methanol was added 1.7 g (12 mmol) of K_2CO_3. After 1 h, the mixture was poured into 100 mL of 0.5 *M* HCl and extracted with ethyl acetate (3 X 100 mL). The combined organic layers were washed successively with water (2 X 100 mL) and saturated NaCl (2 X 100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to afford 4.85 g of crude diol **15**, which was used in the next step without further purification.

To a mixture of 4.85 g of crude **15** and 2.4 g (28 mmol) of 3,4-dihydro-2*H*-pyran in 75 mL of CH_2Cl_2 at 0 °C was added 370 mg (1.9 mmol) of *p*-toluenesulfonic acid monohydrate. After stirring for 45 min, the reaction was poured into 40 mL of saturated $NaHCO_3$, layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 X 40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was chromatographed on silica gel eluting with 40% ethyl acetate in hexanes, to afford 6.0 g (100%) of **16** as an oil. ^{1}H NMR ($CDCl_3$) δ (characteristic peaks only) 7.25-7.14 (m, 1 H), 6.95-6.87 (m, 2 H), 6.83-6.72 (m, 1 H), 5.8-5.4 (m, 4 H), 5.1-4.8 (m, 2 H).

F: (13*E*)-(9*S*, 11*R*, 15*R*)-11,15-Bis(tetrahydropyran-2-yloxy)-16-(3-chlorophenoxy)-2,3,4,5,6,17,18,19,20-nonanor-9-triethylsilyloxy-13-prostenol Triethylsilyl Ether (**18**)

To a suspension of 400 mg (10.5 mmol) of lithium aluminum hydride in 20 mL of THF at 0 °C was added dropwise a solution of 4.5 g (8.8 mmol) of lactone **16** in 20 mL of THF. After 1 h at 0 °C the mixture was cautiously poured into 100 mL of a 1/1 mixture of ice-cold saturated NH_4Cl/ethyl acetate. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 X 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to afford 4.5 g (100%) of diol **17** which was used in the next step without further purification.

12

Triethylsilyl chloride (3.0 g, 20 mmol) was added to a mixture of 4.5 g (8.8 mmol) of crude **17**, 40 mL of DMF, 1.85 g (27.0 mmol) of imidazole, and 310 mg (2.5 mmol) of 4-(dimethylamino)pyridine. After 2 h, the reaction was poured into 100 mL of a 1/1 mixture of ethyl acetate/saturated NH_4Cl, layers were separated, and the aqueous layer was extracted with ethyl acetate (2 X 25 mL). The combined organic layers were washed with water (3 X 25 mL), dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel eluting with 20% ethyl acetate in hexane to afford 5.2 g (80%) of **18**. ^1H NMR ($CDCl_3$) δ (characteristic peaks only) 7.22-7.12 (m, 1 H), 6.95-6.88 (m, 2 H), 6.83-6.71 (m, 1 H), 5.8-5.4 (m, 4 H), 5.1-4.8 (m, 2 H), 1.0-0.85 (m, 18 H), 0.7-0.5 (m, 12 H).

G: (13*E*)-(*9S*, 11*R*, 15*R*)-11,15-Bis(tetrahydropyran-2-yloxy)-16-(3-chlorophenoxy)-2,3,4,5,6,17,18,19,20-nonanor-9-triethylsilyloxy-13-prostenal (**19**)

To a mixture of 1.6 g (12.6 mmol) of oxalyl chloride and 15 mL of CH_2Cl_2 at -78 °C was added dropwise a solution of 1.54 g (19.7 mmol) of DMSO in 2 mL of CH_2Cl_2 . After 10 min, 4.6 g (6.2 mmol) of bissilane **18** in 8 mL of CH_2Cl_2 was added dropwise. After 95 min, 3.0 g (30 mmol) of NEt_3 was added. The mixture was then warmed to room temperature and poured into 70 mL of saturated NH_4Cl. The solution was extracted with of CH_2Cl_2 (3 X 70 mL) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was chromatographed on silica gel eluting with 20% ethyl acetate in hexane to afford 2.06 g (53%) of **19** as well as 1.5 g (26%) recovered **18**. ^1H NMR ($CDCl_3$) δ (characteristic peaks only) 9.78 (t, J = 1.4 Hz, 1 H), 7.22-7.12 (m, 1 H), 6.95-6.88 (m, 2 H), 6.83-6.71 (m, 1 H), 5.8-5.4 (m, 4 H) 5.1-4.8 (m, 2 H), 1.0-0.85 (m, 18 H), 0.7-0.5 (m, 12 H).

H: (5*Z*, 13*E*)-(9*S*, 11*R*, 15*R*)-11,15-Bis(tetrahydropyran-2-yloxy)-16-(3-chloro-phenoxy)-2,3,4,17,18,19,20-heptanor-9-triethylsilyloxy-5,13-prostadienoic Acid Methyl Ester (**21**)

To a solution of 1.35 g (4.24 mmol) of phosphonate **20** and 2.60 g (9.84 mmol) of 18-crown-6 in 20 mL of THF at -78 °C was added dropwise 6.9 mL (3.45 mmol) of a 0.5 *M* solution of potassium hexamethyldisilazane in toluene. After stirring for 15 min, a solution of 1.65 g (2.64 mmol) of aldehyde **19** in 20 mL of THF was added dropwise. One hour later, the mixture was poured into 100 mL of saturated NH_4Cl/ethyl acetate, 1/1, layers were separated, and the aqueous layer was extracted with ethyl acetate (3 X 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated and the residue was chromatographed on silica gel eluting with 20% ethyl acetate in hexane to afford 1.135 g (63%) of **21**. ^1H NMR ($CDCl_3$) δ (characteristic peaks only) 7.22-7.11 (m, 1 H), 6.97-6.86 (m, 2 H), 6.85-6.75 (m, 1 H), 6.4-6.2 (m, 1 H), 5.8-5.32 (m, 3 H), 3.66 (s, 3 H).

I: (5*Z*, 13*E*)-(9*S*, 11*R*, 15*R*)-11,15-Bis(tetrahydropyran-2-yloxy)-16-(3-chloro-phenoxy)-2,3,4,17,18,19,20-heptanor-9-triethylsilyloxy-5,13-prostadien-1-ol (**22**)

To a solution of 850 mg (1.25 mmol) of ester **21** in 10 mL of THF at 0 °C was added 2.4 mL (3.6 mmol) of a 1.5 *M* solution of diisobutylaluminum hydride in toluene. After 1 h, the mixture was poured into 20 mL of saturated NH_4Cl and was extracted with ethyl acetate (3 X 20 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated down to 800 mg (98%) of **22** as an oil. ^1H NMR ($CDCl_3$) δ (characteristic peaks only) 7.25-7.15 (m, 1 H), 6.97-6.90 (m, 2 H), 6.86-6.75 (m, 1 H), 5.81-5.41 (m, 4 H).

J: (5*Z*, 13*E*)-(9*S*, 11*R*, 15*R*)-11,15-Bis(tetrahydropyran-2-yloxy)-16-(3-chloro-phenoxy)-3-oxa-17,18,19,20-tetranor-9-triethylsilyloxy-5,13-prostadienoic Acid Isopropyl Ester (**23**)

To a solution of 415 mg (6.37 mmol) of alcohol **22** in 4 mL of THF at -78 °C was added dropwise 0.35 mL (0.87 mol) of a 2.5 *M* solution of *n*-BuLi in hexane. After 15 min, this solution was transferred *via* syringe to a -78 °C solution of 195

mg (1.08 mmol) of isopropyl bromoacetate in 2 mL of THF. The mixture was kept at -78 °C for 40 min, warmed to room temperature overnight, and then poured into 20 mL of a 1/1 mixture of saturated NH_4Cl/ethyl acetate. Layers were separated, and the aqueous layer was extracted with ethyl acetate (2 X 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and the residue was chromatographed on silica gel (20% ethyl acetate in hexane) to afford 242 mg (53%) of **23** as an oil. ^1H NMR ($CDCl_3$) δ (characteristic peaks only) 7.24-7.15 (m, 1 H), 6.97-6.90 (m, 2 H), 6.86-6.75 (m, 1 H), 5.81-5.41 (m, 4 H), 1.57 (d, J = 5.7 Hz, 6 H).

K: (5*Z*, 13*E*)-(9*S*, 11*R*, 15*R*)-16-(3-Chlorophenoxy)-3-oxa-17,18,19,20-tetranor-9,11,15-trihydroxy-5,13-prostadienoic Acid Isopropyl Ester (**5**)

To a solution of 230 mg (0.32 mmol) of silane **23** in 5 mL of THF at room temperature was added 0.33 mL (0.33 mmol) of a 1 *M* solution of Bu_4NF in THF. After 20 min, the reaction was poured into 4 mL of saturated NH_4Cl and was extracted with ethyl acetate (4 X 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and the residue was chromatographed on silica gel (ethyl acetate/hexane, 1/1), to afford 126 mg (65%) of desilylated compound **24**.

To 120 mg of **24** in 5 mL of methanol was added 0.4 mL of 2 *M* HCl. After 1 h, the mixture was added to 3 mL of saturated $NaHCO_3$, and the resulting mixture was extracted with ethyl acetate (3 X 8 mL). Combined organic layers were dried over $MgSO_4$, filtered, concentrated. The resulting residue was then chromatographed on silica gel eluting with ethyl acetate to afford 54 mg (56%) of **5**. ^{13}C NMR ($CDCl_3$) δ 169.92 (C), 159.26 (C), 135,13 (CH), 134.95 (CH), 134.81 (C), 124.93 (CH), 121.22 (CH), 115.06 (CH), 113.08 (CH), 77.75 (CH), 72.02 (CH), 71.94 (CH_2), 70.76 (CH_2), 68.77 (CH), 67.78 (CH_2), 66.50 (CH_2), 55.46 (CH), 49.93 (CH), 42.47 (CH_2), 25.85 (CH_2), 21.75 (CH_3). CI MS, *m/z* calcd. for $C_{24}H_{34}O_7Cl_1$ (MH^+), 469.1993, found 469.1993.

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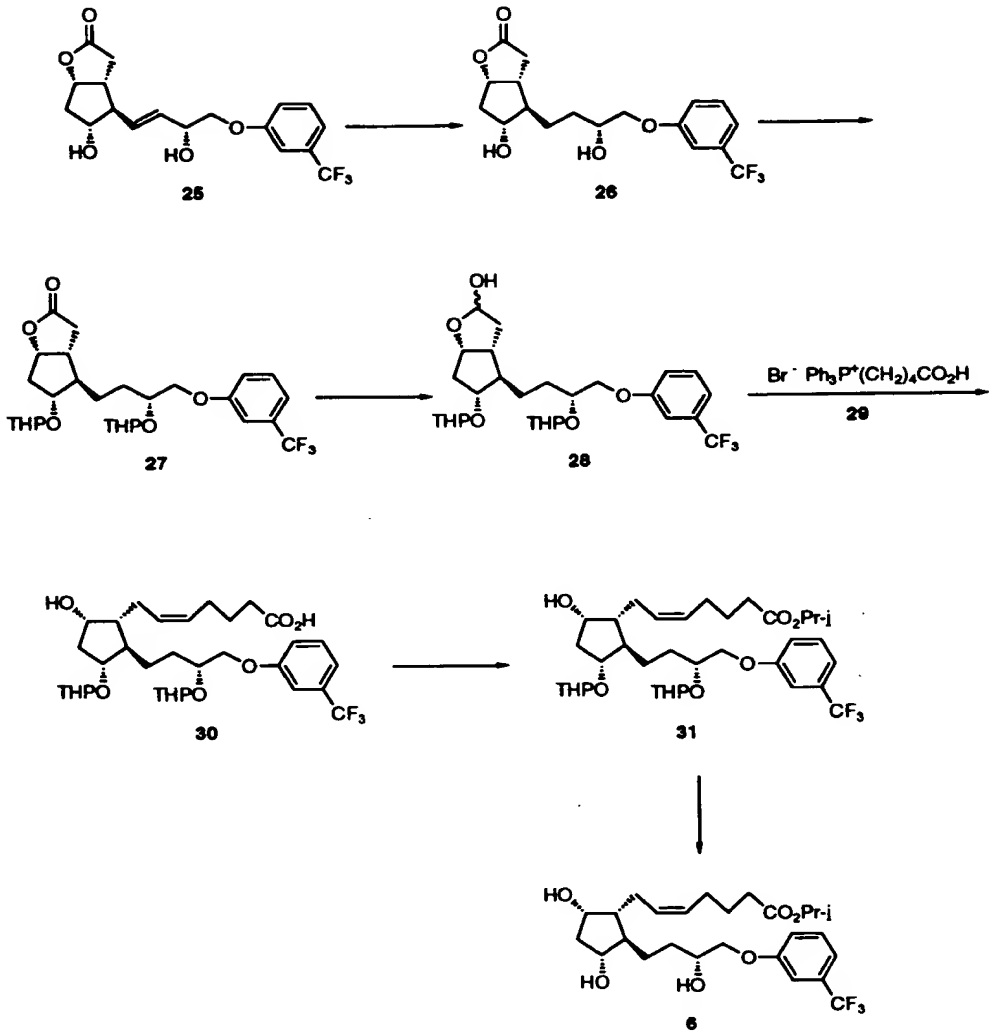
EXAMPLE 2: Synthesis of 13,14-Dihydrofluprostenol Isopropyl Ester



A: (3a*R*, 4*R*, 5*R*, 6a*S*)-5-Hydroxy-4-[(3*R*)-4-(3-trifluoromethylphenoxy)-3-hydroxy-1-butyl]-hexahydro-2*H*-cyclopenta[b]furan-2-one (**26**)

A mixture of 1.2 g (3.2 mmol) of diol **25** (for synthesis of diol **25**, see U.S. Patent 4,321,275) and 0.05 g of 10% (wt/wt) Pd/C in 20 mL of methanol was hydrogenated at 30 psi for 1.5 hours. After filtration through a short pad of Celite® concentration afforded 1.2 g (100%)of **26** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.44 (m, 2 H), 7.12 (m, 2 H), 4.95 (dt, 1 H), 4.15-3.80 (m, 4 H), 2.82 (dd, J = 10.8, 1 H), 2.55 (m, 2 H), 2.3 (m, 1 H), 2.1-1.3 (m, 6 H).

B: (3a*R*, 4*R*, 5*R*, 6a*S*)-5-(Tetrahydropyran-2-yloxy)-4-[(3*R*)-4-(3-trifluoromethylphenoxy)-3- (tetrahydropyran-2-yloxy)-1-butyl]-hexahydro-2*H*-cyclopenta[b]furan-2-one (**27**)

A mixture of 1.2 g (3.2 mmol) of diol **26** and 0.05 g of *p*-toluenesulfonic acid monohydrate in 100 mL of CH_2Cl_2 at 0 °C was treated with 3,4-dihydro-2*H*-pyran (1.1 ml, 12 mmol) and the solution was stirred for 2 h at 0 °C. After pouring into saturated $NaHCO_3$, phases were separated and the organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by chromatography on silica gel (1/1, hexanes/ EtOAc) to afford 1.1 g of **27** as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 8.04 (dd, J = 7.0, 1.6, 1 H), 7.44 (m, 2 H), 7.12 (m, 1 H), 4.95 (dt, 1 H), 4.8 (m, 1 H), 4.7 (m, 2 H), 4.15-3.80 (m, 4 H), 3.5 (m, 2 H), 2.82 (dd, J = 10.8, 1 H), 2.55 (m, 2 H), 2.3 (m, 1 H), 2.1-1.3 (m, 6 H).

C: (5*Z*)-(9*S*, 11*R*, 15 *R*)-11,15-*Bis*(tetrahydropyran-2-yloxy)-9-hydroxy-17,18,19,20-tetranor-16-(3-trifluoromethylphenoxy)-5-prostenoic Acid Isopropyl Ester (**31**)

To a solution of 2.1 g (3.9 mmol) of **27** in 100 mL of THF at -78 °C was added 3.9 mL (5.8 mmol) of a 1.5 M solution of diisobutyaluminum hydride in toluene. The solution was stirred for 2 h, then quenched by the sequential addition of 0.4 mL of isopropanol at -78 °C followed by 0.4 mL of water at 23 °C. Volatiles

17

were removed under reduced pressure and the aqueous solution was extracted with Et_2O/EtOAc (1/1). Organic extracts were dried over $MgSO_4$, filtered, and concentrated to furnish 1.9 g of lactol **28**.

To a 250 mL 3-necked round bottom flask equipped with a mechanical stirrer and a thermometer were added anhydrous DMSO (100 mL) and NaH (80% dispersion in mineral oil; 0.48 g, 16 mmol). The mixture was heated to 75 °C (internal) for 30 min, after which it was allowed to cool to room temperature for 1 h. Phosphonium bromide **29** (3.5 g, 8 mmol) was then added. After stirring for 30 minutes, 1.9 g (3.5 mmol) of lactol **28** in 50 mL of DMSO was added, and the resulting solution was heated to 50 °C for 2 h and then brought to room temperature for 16 h. The solution was poured into 100 mL of water and approximately 2 mL of 50% NaOH added. The aqueous phase was extracted with ether (3 X 100 mL), then made acidic (pH = 5.5) by the addition of a 10% citric acid solution, and extracted with Et_2O/hexanes, 2/1 (3 X 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to afford 1.9 g of **30** as a colorless oil.

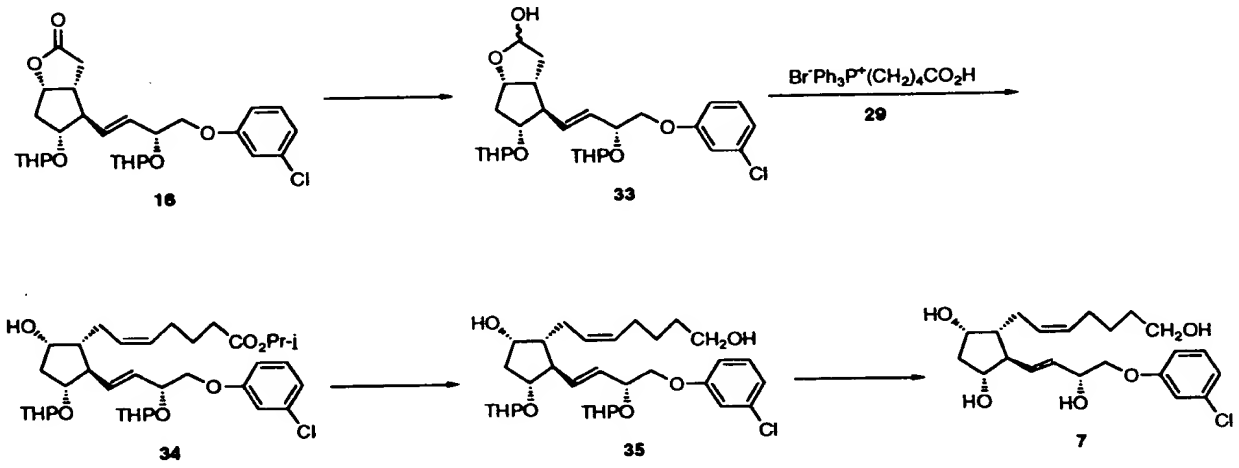
To 1.9 g of carboxylic acid **30** dissolved in 10 mL acetone was added 0.95 g (6.0 mmol) of DBU and 1.0 g (6.1 mmol) of isopropyl iodide at 23 °C. After 16 h, the solution was poured into 100 mL of water and extracted with 100 mL of EtOAc. The organic extract was dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (3/2, hexanes/EtOAc) to afford 1.9 g of isopropyl ester **31** as a colorless oil. ^1H NMR ($CDCl_3$) δ 7.44 (t, 1 H), 7.12 (d, 1 H), 7.12 (dd, 2 H), 5.5-5.3 (m, 2 H), 4.99 (heptet, 1 H), 4.15-3.80 (m, 4 H), 2.82 (dd, J = 10.8, 1 H), 2.55 (m, 2 H), 2.3 (m, 1 H), 2.1-1.3 (m, 24 H), 1.23 (s, 3 H), 1.20 (s, 3 H).

D: (5*Z*)-(9*S*, 11*R*, 15*R*)-17,18,19,20-Tetranor-16-(3-trifluoromethylphenoxy)-9,11,15-trihydroxy-5-prostenoic Acid Isopropyl Ester (6)

Ester **31** (1.9 g, 2.8 mmol) was dissolved in 14 mL of a mixture of AcOH/THF/H_2O (4/2/1) and the solution was heated to 50 °C for 1 h, allowed to cool to 23 °C, poured into a saturated solution of $NaHCO_3$, and extracted with Et_2O

(2 X 100 mL) and EtOAc (100 mL). The combined organic extracts were dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (1/1, hexanes/EtOAc) to furnish 0.5 g of triol **6** as a clear, colorless oil. ^1H NMR ($CDCl_3$) δ 7.44 (t, J = 7.8, 1 H), 7.12 (dd, J = 7.8, 2.0, 1 H), 7.12 (ddd, J = 15.6, 7.2, 2.0, 2 H), 5.5-5.3 (m, 2 H), 4.99 (heptet, J = 6.3, 1 H), 4.15-3.80 (m, 4 H), 3.2 (d, 1 H), 2.95 (s, 1 H), 2.82 (dd, J = 10.8, 1 H), 2.75 (d, J = 5.9, 1 H), 2.55 (m, 2 H), 2.3 (m, 1 H), 2.1-1.3 (m, 24 H), 1.23 (s, 3 H), 1.20 (s, 3 H). ^{13}C NMR ($CDCl_3$) δ 173.5, 158.7, 132.1, 131.5, 130.0, 129.5, 129.2, 123.3, 120.8, 117.7, 117.6, 111.4, 111.4, 78.6, 74.4, 72.4, 69.9, 67.6, 52.6, 51.7, 42.5, 34.0, 31.5, 29.4, 26.8, 26.6, 24.9, 21.7.

EXAMPLE 3: Synthesis of Cloprostenol-1-ol (7)



A: (5*Z*, 13*E*)-(9*S*, 11*R*, 15*R*)-11,15-Bis(tetrahydropyran-2-yloxy)-16-(3-chlorophenoxy)-9-hydroxy-17,18,19,20-tetranor-5,13-prostadienoic Acid Isopropyl Ester (**34**)

A 1.5 *M* solution of diisobutylaluminum hydride in toluene (10 mL, 15 mmol) was added dropwise to a solution of 5.8 g (11.4 mmol) of lactone **16** in 55 mL of THF at -78 °C. After 1 h, 10 mL of methanol was added dropwise, and the mixture

was stirred for 10 min at -78 °C before being warmed to room temperature. The mixture was then poured into 100 mL of a 1/1 solution of saturated aqueous potassium sodium tartrate/ethyl acetate and stirred. After separating layers, the aqueous phase was extracted with ethyl acetate (2 X 40 mL). Combined organic layers were dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (3/2, ethyl acetate/hexane), to afford 4.4 g (76%) of lactol **33**, which was used immediately in the next step.

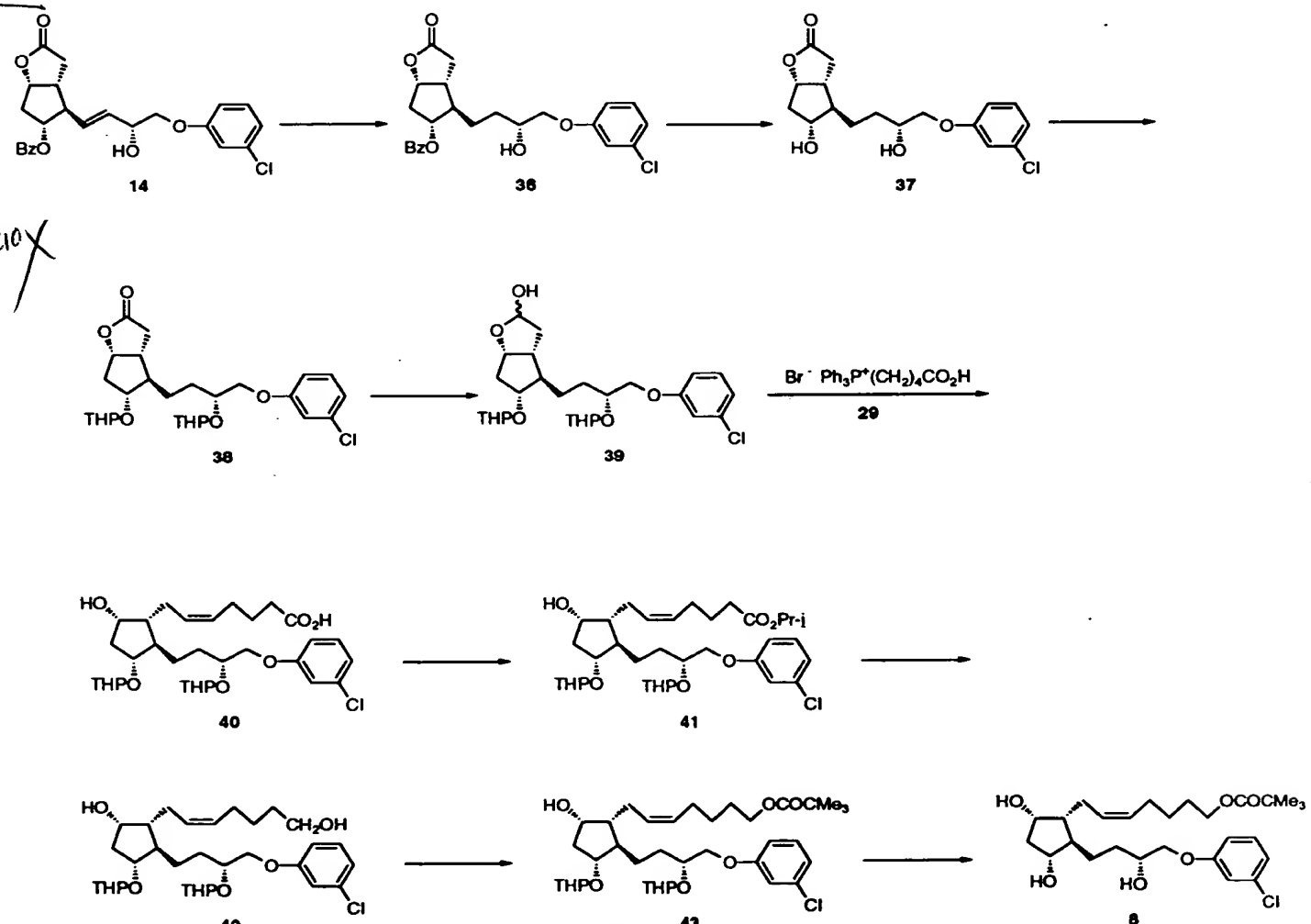
A 1 *M* solution of potassium *t*-butoxide in THF (50.0 ml) was added dropwise to 12.1 g (27.3 mmol) of phosphonium salt **29** in 100 mL of THF at 0 °C. After 30 min, a solution of 4.4 g (8.6 mmol) of lactol **33** in 20 mL of THF was added dropwise, and the mixture was stirred at room temperature overnight. The solution was then poured into 150 mL of a 1/1 mixture of ethyl acetate/saturated NH_4Cl. Layers were separated and the aqueous layer was extracted with ethyl acetate (2 X 100 mL). Combined organic layers were dried over $MgSO_4$, filtered, concentrated, and the residue was redissolved in 80 mL of acetone. To this was added 6.5 g (45 mmol) of DBU followed by 7.3 g (43 mmol) of isopropyl iodide. After stirring overnight, the reaction was poured into 100 mL of a 1/1 mixture of ethyl acetate/saturated NH_4Cl. Layers were then separated and the aqueous phase was further extracted with ethyl acetate (2 X 100 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (40% ethyl acetate in hexane) to afford 2.92 g (53% from lactone **16**) of ester **34**.

B: (5*Z*, 13*E*)-(9*S*, 11*R*, 15*R*)-16-(3-Chlorophenoxy)-17,18,19,20-tetranor-9,11,15-trihydroxy-5,13-prostadienol (**7**)

A solution of 500 mg (0.79 mmol) of **34** in 10 mL of THF was added dropwise to 61 mg (1.60 mmol) of lithium aluminum hydride in 20 mL of THF at 0 °C. After 40 min, the reaction was carefully poured into 15 mL of saturated NH_4Cl, and the mixture was then extracted with ethyl acetate (3 X 40 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated to afford 500 mg of crude **35**.

To a solution of 500 mg of **35** in 20 mL of methanol was added 0.5 mL of 2 *M* HCl. After 1 h, the reaction was quenched with 20 mL of saturated $NaHCO_3$ and the mixture was extracted with ethyl acetate (4 X 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel chromatography (EtOAc) provided 101 mg (31% from **34**) of **7**. ^{13}C NMR ($CDCl_3$) δ 159.27 (C), 135.44 (CH), 134.82 (C), 130.64 (CH), 130.26 (CH), 128.23 (CH), 121.25 (CH), 115.07 (CH), 113.08 (CH), 77.35 (CH), 72.35 (CH), 71.90 (CH_2), 70.89 (CH), 62.22 (CH_2), 55.40 (CH), 49.87 (CH), 42.79 (CH_2), 31.83 (CH_2), 26.77 (CH_2), 25.60 (CH_2), 25.33 (CH_2). CI MS *m/z* calcd for $C_{22}H_{32}O_5Cl_1$ (MH^+) 411.1938, found 411.1938.

EXAMPLE 4: Synthesis of 13,14-Dihydrocloprostenol-1-ol Pivaloate (8)



A: (3a*R*, 4*R*, 5*R*, 6a*S*)-4-[(3*R*)-4-(3-Chlorophenoxy)-3-hydroxybutyl]-5-hydroxy-hexahydro-2*H*-cyclopenta[b]furan-2-one (**37**):

A mixture of 2.4 g (5.4 mmol) of **14** and 250 mg of 10% (wt/wt) Pd/C in 35 mL of ethyl acetate was hydrogenated at 40 psi for 1 h. After filtration through a short pad of Celite®, the filtrate was evaporated down to 2.3 g (100%) of hydrogenated product **36**.

The crude benzoate **36** was dissolved in 25 mL of methanol, and 610 mg (4.4 mmol) of K_2CO_3 was added. After 3.5 h, the mixture was poured into 100 mL of water/ethyl acetate (1/1). Layers were separated, and the aqueous phase was further extracted with ethyl acetate (2 X 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Silica gel chromatography (EtoAc) provided 1.50 g (82%) of **37** as a white solid, m.p. = 102.0-103.5 °C. ^1H NMR δ 7.22 (t, J = 8.2 Hz, 1 H), 7.0-6.94 (m, 1 H), 6.91-6.88 (t, J = 2.1 Hz, 1 H), 6.83-6.77 (m, 1 H), 4.97 (dt, J = 3.0, 8.3 Hz, 1 H), 4.12-3.91 (m, 3 H), 3.82 (dd, J = 7.4, 9.0 Hz, 1 H), 2.85 (dd, J = 8.0, 16.5 Hz, 1 H), 2.6-1.4 (m, 11 H).

B: (3a*R*, 4*R*, 5*R*, 6a*S*)-4-[(3*R*)-4-(3-Chlorophenoxy)-3-(tetrahydropyran-2-yloxy)butyl]-5-(tetrahydropyran-2-yloxy)-hexahydro-2*H*-cyclopenta[b]furan-2-one (**38**)

Diol **37** (3.4 g, 10 mmol) and 2.2 g (26 mmol) of 3,4-dihydro-2*H*-pyran were dissolved in 80 mL of CH_2Cl_2, and 240 mg (1.3 mmol) of *p*-toluenesulfonic acid monohydrate was added at 0 °C. After 1 h, the reaction was poured into 50 mL of saturated $NaHCO_3$ and the mixture was extracted with CH_2Cl_2 (3 X 40 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and the residue was chromatographed on silica gel (hexane/ethyl acetate, 1/1) to afford 4.5 g (87%) of bis-THP ether **38**.

C: (5*Z*)-(9*S*, 11*R*, 15*R*)-11,15-Bis(tetrahydropyran-2-yloxy)-16-(3-chlorophenoxy)-9-hydroxy-17,18,19,20-tetranor-5-prostenoic Acid Isopropyl Ester (**41**)

A 1.5 *M* solution of diisobutylaluminum hydride in toluene (1.8 mL, 2.7 mmol) was added to the solution 1.05 g (2.06 mmol) of **38** in 10 mL of THF at -78 °C. After 1 h, 4 mL of methanol was added and the mixture was warmed to 25 °C, then

poured into 40 mL of ethyl acetate/saturated aqueous potassium sodium tartrate (1/1). Layers were separated and the aqueous phase was further extracted with ethyl acetate (3 X 30 mL). The combined organic layers were then dried over $MgSO_4$, filtered, concentrated, and the residue was chromatographed on silica gel (ethyl acetate) to afford 740 mg (70%) of lactol **39**.

A 1.5 *M* solution of potassium *t*-butoxide in THF (8.6 mL, 8.6 mmol) was added dropwise to a mixture of 15 mL of THF and 1.92 g (4.33 mmol) of phosphonium salt **29** at 0 °C. After stirring for 1 h, a solution of 740 mg (1.45 mmol) of lactol **39** in 5 mL of THF was added dropwise, and the reaction was allowed to warm to 25 °C overnight. The mixture was then poured into 100 mL of ethyl acetate/saturated NH_4Cl (1/1). Layers were separated, and the aqueous phase was further extracted with ethyl acetate (2 X 70 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated to afford 1.6 g of crude acid **40**.

Crude acid **40** (1.6 g) was dissolved in 11 mL of acetone and cooled to 0 °C, then 850 mg (5.6 mmol) of DBU was added dropwise to the solution. The resulting mixture was stirred for 15 min at 0 °C and 30 min at 25 °C, after which 850 mg (5.0 mmol) of isopropyl iodide was added. The reaction was stirred overnight and poured into 100 mL of ethyl acetate/saturated NH_4Cl (1/1). Layers were separated, and the aqueous phase was further extracted with ethyl acetate (2 X 50 mL). Combined organic layers were dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by silica gel chromatography (ethyl acetate/hexanes, 3/2) to afford 560 mg (61% from lactol **39**) of isopropyl ester **41**.

D: (5*Z*)-(9*S*, 11*R*, 15*R*)-16-(3-Chlorophenoxy)-17,18,19,20-tetranor-9,11,15-trihydroxy-5-prostenol Pivaloate (**8**)

A solution of 400 mg (0.63 mmol) of **41** in 5 mL of THF was added dropwise to a suspension of 35 mg (0.92 mmol) of lithium aluminum hydride in 5 mL of THF at 0 °C. After 2 h, the reaction was poured into 50 mL of a 1/1 mixture of ethyl acetate/saturated $NaHCO_3$. The layers were then separated, and the aqueous phase was extracted with ethyl acetate (2 X 2 mL). Combined organic layers were

dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (ethyl acetate) to afford 350 mg (95%) of diol **42**.

Pivaloyl chloride (90 mg, 0.75 mmol) was added to a mixture of 350 mg (0.60 mmol) of **42**, 60 mg (0.76 mmol) of pyridine, 22 mg (0.18 mmol) of 4-(dimethylamino)pyridine, and 7 mL of CH_2Cl_2. After 1.5 h, the mixture was poured into 30 mL of saturated NH_4Cl/ethyl acetate (1/1). Layers were then separated and the aqueous phase was extracted with ethyl acetate (2 X 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (ethyl acetate/hexane, 3/2) to afford 370 mg (93%) of pivaloate **43**.

Water (approximately 10 drops) and concentrated HCl (approximately 3 drops) were added to a solution of 370 mg (0.56 mmol) of **43** in 5 mL of methanol. After stirring overnight, the reaction was quenched by the addition of 20 mL of saturated $NaHCO_3$, and the mixture was extracted with ethyl acetate (3 X 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was chromatographed on silica gel (ethyl acetate/hexane, 3/2), to afford 165 mg (59%) of triol **8**. ^{13}C NMR ($CDCl_3$) δ 178.77 (C), 159.27 (C), 134.80 (C), 130.20 (CH), 128.62 (CH), 121.19 (CH), 114.97 (CH), 112.97 (CH), 78.50 (CH), 74.46 (CH), 72.31 (CH_2), 69.86 (CH), 64.16 (CH_2), 52.53 (CH), 51.67 (CH), 42.50 (CH_2), 31.51 (CH_2), 29.40 (CH_2), 28.10 (CH_2), 27.12 (CH_3), 26.77 (CH_2), 26.65 (CH_2), 25.77 (CH_2). CI MS, *m/z* calcd for $C_{27}H_{41}O_6Cl_1$ (MH^+), 497.2670, found 497.2656

EXAMPLE 5

$PGF_{2\alpha}$ analogues are known to contract the iris sphincter of cats and this assay is a generally accepted reference for activity. For this reason, the pupil diameter of cats may be used to define the activity of $PGF_{2\alpha}$ analogues and, as demonstrated by Stjernschantz and Resul (Drugs Future, 17:691-704 (1992)), predict the IOP-lowering potency.

24

Compounds of the present invention were therefore screened for pupillary constriction in the cat. Data for compounds **6, 7,** and **8** are presented in Table 2, below. The response is quantitated as Area $_{1\text{-}5}$ values (area under the pupil diameter versus time curve from 1-5 hours), and the equivalent response dose (ED_5) is estimated from its dose response relationship.

Table 2: Cat Pupil Diameter Response

Compound	ED_5 (µg)
$PGF_{2\alpha}$ Isopropyl Ester	0.02
Cloprostenol Isopropyl Ester	0.01
6	0.2
7	0.02
8	0.06

Discussion:

The two standard compounds, $PGF_{2\alpha}$ isopropyl ester and cloprostenol isopropyl ester, produced marked change in cat pupillary diameter, displaying ED_5 values of 0.02 and 0.01 µg, respectively. Compound **7** (cloprostenol-1-ol) and compound **8** (13,14-dihydrocloprostenol-1-ol pivaloate), displayed nearly equivalent potency. 13,14-Dihydrofluprostenol isopropyl ester (compound **6**) was approximately one order of magnitude less potent, with an ED_5 of 0.2 µg.

EXAMPLE 6

In the study presented below, compound **6** (Table 1, above) was tested for IOP-lowering effect in cynomolgus monkey eyes.

The right eyes of the cynomolgus monkeys used in this study were previously given laser trabeculoplasty to induce ocular hypertension in the lasered eye. Animals had been trained to sit in restraint chairs and conditioned to accept experimental procedures without chemical restraint. IOP was determined with a

pneumatonometer after light corneal anesthesia with dilute proparacaine. The test protocol included a five-dose treatment regimen because of the typical delayed response to prostaglandins. The designated test formulations were administered to the lasered right eyes, and the normal left eyes remained untreated, although IOP measurements were taken. Baseline IOP values were determined prior to treatment with the test formulation, and then IOP was determined from 1 to 7 hours after the first dose, 16 hours after the fourth dose, and 1 to 4 hours after the fifth dose.

The equivalent response dose (ED_{20}) is estimated from the dose response relationship to be the dose producing a 20% peak reduction in IOP.

Table 3: Monkey IOP Response

Compound	ED_{20} (µg)
$PGF_{2\alpha}$ Isopropyl Ester	0.4
6	0.3

Discussion:

As can be seen in Table 3, compound **6**, the 13,14-dihydro analogue of fluprostenol was quite potent in the monkey IOP model, producing a 20% reduction at 0.3 µg. This was even more potent than the standard compound, $PGF_{2\alpha}$ isopropyl ester.

EXAMPLE 7

The following Formulations 1-4 are representative pharmaceutical compositions of the invention for topical use in lowering of intraocular pressure. Each of Formulations 1 through 4 may be formulated in accordance with procedures known to those skilled in the art.

FORMULATION 1

Ingredient	Amount (wt%)
Compound **5** (Table 1)	0.002
Dextran 70	0.1
Hydroxypropyl methylcellulose	0.3
Sodium chloride	0.77
Potassium chloride	0.12
Disodium EDTA	0.05
Benzalkonium chloride	0.01
HCl and/or NaOH	pH 7.2 - 7.5
Purified water	q.s. to 100%

FORMULATION 2

Ingredient	Amount (wt%)
Compound **6** (Table 1)	0.01
Monobasic sodium phosphate	0.05
Dibasic sodium phosphate (anhydrous)	0.15
Sodium chloride	0.75
Disodium EDTA	0.01
Benzalkonium chloride	0.02
Polysorbate 80	0.15
HCl and/or NaOH	pH 7.3 - 7.4
Purified water	q.s. to 100%

FORMULATION 3

Ingredient	Amount (wt%)
Compound **7** (Table 1)	0.001
Dextran 70	0.1
Hydroxypropyl methylcellulose	0.5
Monobasic sodium phosphate	0.05
Dibasic sodium phosphate (anhydrous)	0.15
Sodium chloride	0.75
Disodium EDTA	0.05
Benzalkonium chloride	0.01
NaOH and/or HCl	pH 7.3 - 7.4
Purified water	q.s. to 100%

FORMULATION 4

Ingredient	Amount (wt%)
Compound **8** (Table 1)	0.003
Monobasic sodium phosphate	0.05
Dibasic sodium phosphate (anhydrous)	0.15
Sodium chloride	0.75
Disodium EDTA	0.05
Benzalkonium chloride	0.01
HCl and/or NaOH	pH 7.3 - 7.4
Purified water	q.s. to 100%

The invention has been described by reference to certain preferred embodiments; however, it should be understood that it may be embodied in other specific forms or variations thereof without departing from its spirit or essential characteristics. The embodiments described above are therefore considered to be illustrative in all respects and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description.